Article

Chinese Science Bulletin

Pharmacy

November 2013 Vol.58 No.33: 4092–4100
doi: 10.1007/s11434-013-5861-8

A quality evaluation strategy for *Rhizoma coptidis* from a variety of different sources using chromatographic fingerprinting combined with biological fingerprinting

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Received October 2, 2012; accepted March 7, 2013; published online July 22, 2013

Most of herbal medicines of different sources are used for the same treatment goal in clinic despite their tremendous differences of the quality. Because the clinical efficacy is closely related to the quality of medicines, the implementation of an effective quality control (QC) process is vital. Chemical methods based on content determination and chromatographic fingerprints (CFs) have been recommended as powerful tools. In spite of advances in QC, it is still difficult to determine that which of the chemical compositions can best reflect the correlation with the desired biological activity. Herein, we have focused on the biological activities of multi-source herbal medicines to establish an analytical method. By looking for correlations between the biological activities and their chemical compositions, the method can identify reasonable chemical indicators or chemical methods with biological activity information. In this study, high-performance liquid chromatography (HPLC) and microcalorimetry are combined as a useful method for evaluating the quality of *Rhizoma coptidis* from different sources. The results of biological fingerprints (BFs) showed that the antibacterial activity sequence of *R. coptidis* of different sources was *Coptis teeta* Wall. (*CT*)>*Coptis chinensis* Franch. (*CC*)>*Coptis deltoidea* C.Y. Cheng et Hsiao (*CD*). The "spectrum-activity" relationships between CFs and BFs were investigated using the canonical correlation analysis (CCA). Compared with the CCA result of CFs and BFs, the canonical correlation coefficient r_1 and r_2 were 0.9974 and 0.9752, respectively. It revealed that there are a close correlation between the spectrum-activity relationships and several of the major anti-bacterial components in the CFs, including jatrorrhizine, coptisine, palmatine, and berberine. Of course, *R. coptidis* was only a representative of multi-source medical plants. The result of the current study will provide an effective reference for evaluating the quality of multi-source herbal medicines.

***Rhizoma coptidis*, multi-source medical plants, quality control, chromatographic fingerprint, biological fingerprint**

Citation: Li J X, Yan D, Ma L N, et al. A quality evaluation strategy for *Rhizoma coptidis* from a variety of different sources using chromatographic fingerprinting combined with biological fingerprinting. Chin Sci Bull, 2013, 58: 4092–4100, doi: 10.1007/s11434-013-5861-8

Most of herbal medicines of different sources are used for the same treatment goal in clinic, but in fact there are some tremendous differences in the quality of these herbal medicines because of differences in their geographic origins, varieties, cultivation methods, picking periods and processing methods. And there were hardly any reports about the differences in the quality of these herbal medicines especially their efficacy. With this in mind, questions about the best way to carry out the quality control (QC) of herbal medicines have attracted considerable and increasing levels of research attention in recent years. Because the clinical

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efficacy is closely related to the quality of herbal medicines, the implementation of an effective QC process is vital to provide references for the clinical medication.

Chromatographic fingerprints (CFs) and the quantitative assay of multiple ingredients represent powerful techniques for the QC for herbal medicines of different sources [1−3]. Increasingly advanced analytical methods continue to be developed and applied in a QC context, including HPLC [4], liquid-chromatography mass spectrometry [5], ultra-performance liquid chromatography [6], and ultra-high-performance liquid chromatography coupled with a triple quadrupole electrospray tandem mass spectrometry [7]. In spite of advances in these techniques, however, the compositions of herbal medicines are particularly complex and it is difficult to detect the compositions of these mixtures comprehensively and effectively correlate these relationships with the biological activities of the materials using the conventional detecting methods. This problem is of great importance to the effective QC for herbal medicines of different sources, especially for herbal medicines in which there are great differences in the clinical efficacy.

Some herbal medicines of different sources, such as *Radix Ginseng*, *Aconite*, *Fritillaria thun-bergli*, *Rhizoma coptidis* and *Akebia quinata*, all have certain biological activities. According to the characteristics of herbal medicines, we can evaluate the quality of multi-source medical plants using the bioassay method. Of course, to reflect the inner quality of multi-source medical plants completely, chemical analysis methods can also be combined with bio-analytical methods to establish a standard that can fully reflect the quality of multi-source herbal medicines by using effective ingredients (or limited compositions) as indicators.

The biological activity of herbal medicines of different sources can be evaluated by detecting changes of the energy, because the biological activity of the herbal medicines is closely related to its energy. With this in mind, microcalorimetry, which is a quantitative and versatile method used for evaluating biothermokinetics in the pharmaceutical analysis [8], biotechnology [9], and environmental sciences, represents a good mean of analyzing the biological activity. This technique has been used successfully to establish biological fingerprints (BFs) for evaluating the effects of different varieties of substances on microbial metabolism [10]. This method is also applicable for investigating dynamic heat metabolism together with the growth processes of an organism. Under suitable conditions, the specific microbial activity can be affected by the different varieties of substances in the plant materials, to the extent that the curves that are directly related to an increase or decrease in the energy release would shift and be different from the standard curves [11]. Microcalorimetry is a highly sensitive and highly accurate method and is also amenable to automation. The technique can also reveal temporal details, such as the thermal effect, which cannot be observed by any of the other techniques [12,13]. The microcalorimetry technique therefore represents a useful tool for the QC of herbal medicines of different sources with biological activities.

Rhizoma coptidis (Huanglian in Chinese) (*R. coptidis*) is a strong and efficacious antibacterial, anti-inflammatory, detoxicant, and antiviral material that is also effective in removing toxicosis and dispelling dampness [14−17]. The antibacterial activity of this herb represents its most pronounced pharmacological effect. From a clinical perspective, the three sources of *R. coptidis* from the dried roots of *Coptis chinensis* Franch. (*CC*), *Coptis deltoidea* C.Y. Cheng et Hsiao (*CD*) and *Coptis teeta* Wall. (*CT*) have been recorded in the Japanese [1] and Chinese Pharmacopoeias [2]. Several methods have been used for the chemical analysis of these materials, including thin-layer chromatography and HPLC. Unfortunately, however, there is no method combining the CFs of these materials with their BFs applied for the quality evaluation of the *R. coptidis* of different sources. In addition, only a limited number of reports based on the applications of biological-assessment methods have been published in the literature [18]. Therefore, it is urgent to establish effective methods for the quality control and evaluation of multi-source medical plants.

In the current study, a feasible approach to evaluate the anti-bacterial activities of *R. coptidis* of different sources was established as a QC method. The approach involved the combination of the chemical analysis (HPLC) with a bioanalytical method (microcalorimetry). *R. coptidis* was selected as a typical representative of multi-source medical plants. The results of the current study will provide an effective reference for evaluating the quality of multi-source medical plants.

1 Experimental

1.1 Reagents and materials

HPLC-grade acetonitrile and methanol were purchased from Fisher Chemicals (Pittsburgh, PA, USA). Water was purified using a Milli-Q Water purification system (Millipore, Bedford, MA, USA). All other chemicals were of analytical grade and purchased from the Beijing Chemical Factory (Beijing, China).

Reference substances containing columbamine (COL), jatrorrhizine (JAT), epiberberine (EPI), coptisine (COP), palmatine (PAL), and berberine (BER) were purchased from the National Institute for Food and Drug Control (NIFDC, Beijing, China). Their purities were >99.8%. *Shigella dysenteriae* (CMCC B 51252) (*S. dysenteriae*) was also obtained from the NIFDC. The fourth-generation *S. dysenteriae* was used in the microcalorimetry assay.

R. coptidis were purchased from the Chongqing, Sichuan, and Yunnan areas of China. The herbs that were identified by Prof. Xiao He Xiao (Institute of Chinese Materia Medica, 302 Hospital, Chinese People's Liberation Army, Beijing, China) were *CD*, *CC*, and *CT*.

1.2 Preparation of culture medium

The nutrient broth culture medium contained beef extract (3 g), NaCl (5 g), and peptone (10 g) in aqueous solution (1000 mL) at pH 7.2–7.4, and was used to cultivate *S. dysenteriae*. The lactose broth (LB) culture medium contained yeast extract (5 g), peptone (10 g), and NaCl (5 g) in aqueous solution (1000 mL) at pH 7.2–7.4, and was used in the microcalorimetry assay.

These broths were sterilized at 121°C under high pressure (0.1 MPa) steam for 15 min, and subsequently preserved at 4°C.

1.3 Extraction procedure

The multi-source *R. coptidis* were initially dried at room temperature and then ground into individual powders. The three different powders were then accurately weighed. The powders were then placed into individual flasks followed by 8 volumes (v/w) of purified water. After a 30-min period of agitation, the mixtures were extracted three times by refluxing extraction, with 1.0 h being allowed for each cycle. The solutions were then filtered while they were still hot and the filtrates collected. Finally, the filtrates were evaporated to dryness to give the desired residues [19].

1.4 Chromatographic fingerprint assay

(i) Preparation of the standard and sample solutions. Standard solutions containing six reference substances were made up by accurately weighing the required amount of each standard material and dissolving it into a volumetric flask with methanol. The standard solutions were then diluted to a series of different concentration for use in the construction of the calibration curves.

The extracts collected from the multi-source *R. coptidis* were accurately weighed (20 mg) before being transferred into a 25-mL volumetric flask and diluted with methanol. The sample solution was then diluted to a series of different final concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg mL^{-1}.

All of the solutions were filtered through a 0.22-μm micropore film (Carrigtwohill Co., Cork, Ireland) prior to their injection into the HPLC system [20].

(ii) Apparatus and conditions. An Agilent 1200 HPLC system (Agilent Technologies, Santa Clara, CA, USA) was used for analyzing the alkaloids from *R. coptidis*. The system consisted of a quaternary pump, vacuum degasser, auto sampler manager, column compartment, and diode array detector (DAD).

A Kromasil™ C_{18} column (250 mm×4.6 mm, 5 μm) operated at 30°C was used for chromatographic separation. The mobile phase consisted of a 1∶1 (v/v) mixture of acetonitrile and an aqueous buffer (0.05 mol L^{-1} potassium dihydrogen phosphate) with sodium dodecyl sulfate also added to the mixture (0.4 g per 100 mL, pH 4.0). The mobile flow rate was set at 0.6 mL min^{-1}. These conditions were taken from the Chinese Pharmacopoeia [2]. The sample injection volume was 10 μL. The detection wavelength was 345 nm. The CF of each sample was recorded in triplicate and the mean values were calculated and used for the analysis.

(iii) Similarity analysis of the CFs. The similarity analysis of the CFs involved the use of a similarity evaluation system for the CFs of traditional Chinese medicines (TCMs, Chinese Pharmacopoeia Committee, Version, 2004A). This software can be used to estimate the similarities and fluctuations in the different chemical materials contained within the samples. The software requires the user to define common peaks and a representative standard chemical fingerprint (RSCF). A RSCF was generated using the mean values from some of the batches of the standard samples. The relative retention time and the relative peak area of common peaks in all samples were calculated from a comparison with the RSCF.

1.5 Microcalorimetry assay

(i) Preparation of sample solution. The different extracts of *R. coptidis* were accurately weighed and dissolved in the LB culture medium and diluted to a series of different final concentrations, including 0.2, 0.4, 0.6, 0.8, 1.0, and 1.2 mg mL^{-1}. Under sterile conditions, 5 mL sample solutions were then added to a 20-mL ampoule which contained 5 mL of 2×10^6 cell mL^{-1} *S. dysenteriae* liquid. A blank control ampoule was also used to inject 5 mL of the L.B. culture medium and 5 mL of a 2×10^6 cell mL^{-1} *S. dysenteriae* liquid.

(ii) Apparatus and conditions. In this study, the metabolic power-time (*p-t*) curves of the growth of *S. dysenteriae* in relation to different concentrations of the *R. coptidis* of different sources were determined using the 3114 TAM air isothermal microcalorimeter (Thermometric AB, Stockholm, Sweden), and the detectable temperature was thermostated at 37°C. The instrument used in the current study was an eight-channel isothermal heat conduction calorimeter operating in the milliwatt range. For more technical information regarding the application of the microcalorimeter, see the report by Wadsö [21].

Each calorimetric channel was constructed in twin configuration with one side for the sample and the other side for a static reference. The ampoules containing sample solution were placed into the sample compartments and another reference ampoule containing sterile water (10 mL) was placed into the other side. The software included with the instrument was used to track and record the *p-t* curves of the growth of *S. dysenteriae*. When the curves returned to the baseline, the experiment was ended. The *p-t* curves could then be mapped using the Origin 8.5 software (OriginLab Corporation, Northampton, MA, USA). Characteristic parameters for the growth of *S. dysenteriae* could also be extracted

using the Origin 8.5 software.

(iii) Similarity analysis of the BFs. According to the similarity analyses conducted on the HPLC CFs of traditional Chinese medicines from a variety of different sources [22], the *p-t* curves of *S. dysenteriae* affected by the different concentrations of the *R. coptidis* of different sources could be evaluated based on their similarities. The similarities of the *p-t* curves were calculated using the vector angle cosine model [23].

(iv) Principal component analysis (PCA). PCA is a variable reduction technique, in which original variables are replaced by latent variables. This technique is a multivariate technique that analyzes a data table in which observations are described by several inter-correlated quantitative dependent variables [24]. In the current study, PCA was undertaken on the multiple variables obtained from the *p-t* curves of all the samples to identify the main variables using SAS statistical software (SAS for Windows 9.1, SAS, Chicago, IL, USA).

1.6 Canonical correlation analysis (CCA)

CCA is a standard tool of multivariate statistical analysis for the discovery and quantification of associations between two sets of variables [25]. The robust determination of the covariance matrix and the associated mean values is essential to the successful application of this modern technique [26]. In the current study, CCA was performed on the many quantitative parameters taken from the CFs and BFs to abstract the main parameters pair of the typical variable and correlation coefficient.

2 Results and discussion

2.1 CF analysis of *R. coptidis*

(i) Method validation. To obtain a stable and repeatable CF of *R. coptidis*, the validation of the method was undertaken on the basis of peak areas and retention times. Under the conditions of the CF, the baseline was stable and completely undisturbed by the methanol solvent. According to the peak areas and retention times of the standard solutions, we could also accurately identify these peaks in the samples. The results are shown in Figure 1.

To confirm the reproducibility, six parallel samples of the same concentration were injected into the HPLC system for six times at 30°C. The relative standard deviations (RSD) for the precision of the peak areas and retention times of all the peaks were ⩽5% and ⩽3%, respectively. The stability test was conducted with the samples following 0, 2, 4, 8, 12 and 24 h at 30°C. The RSD of the peak areas was <4%. The results indicated that the sample solution was stable within a 24-h period. Taken together, the results indicated that the HPLC method for the fingerprint analysis was valid and satisfactory.

(ii) Quantitative analysis of samples. When we compared HPLC CFs of samples from different concentrations of *R. coptidis* of different sources with the standard substances at the same retention time, six of the alkaloids presenting in the 18 samples were identified as being COL, JAT, EPI, COP, PAL and BER. The peak areas of the CFs were tested and recorded. When there is no peak detected, the peak area was recorded as 0.0.

Figure 1(b) shows a number of common peaks in each of *R. coptidis* of different sources. It is interesting to note that the peak area varied significantly from *R. coptidis* of three different sources. The six alkaloids identified only existed in *CC*, whereas *CD* and *CT* did not contain peak 1 (COL). Peak 3 (EPI) could not be detected in the CF of CT. The similarities between the *R. coptidis* of different sources were *CD* 0.953, *CC* 0.979, and *CT* 0.971 (0.3 mg mL^{-1}).

2.2 BF analysis of *R. coptidis*

(i) BF and biothermokinetic parameters. Figure 2 shows the *p-t* curves of *S. dysenteriae* growth at 37°C. The shape of these BFs could be divided into four phases: (1) the first exponential growth phase (A-B); (2) the transition phase (B-C); (3) the second exponential growth phase (C-D); and (4) the decline phase (D-E) [27]. The quantitative biothermokinetic parameters k_1 and k_2 were the growth rate constants of the *S. dysenteriae*. They were obtained by fitting the $\ln p_t$ value of the exponential growth (sections AB and CD in Figure 2) with t to a linear equation according to one of the following equations [9]:

$$p_t = p_0 \exp(kt) \text{ or } \ln p_t = \ln p_0 + kt, \quad (1)$$

where p_0 represents the heat power at the beginning of the baseline, and p_t represents the power at time t. The variables p_1 and p_2 were the maximum heating powers during the first and second stages (peaks B and D in Figure 2). The t_1 and t_2 values were the times to peak of p_1 and p_2. The total generated heat of *S. dysenteriae* Q_t was obtained by calculating the area under the *p-t* curves.

(ii) Similarity analysis. Figure 3 shows the corresponding *p-t* curves of *S. dysenteriae* growth affected by different concentrations and multi-source *R. coptidis*. The influences of the different samples were evaluated using their similarities. In this particular instance, for example, the similarity was introduced with the *p-t* curves of *S. dysenteriae* growth affected by different concentrations of *CD*. Thus, the correlation coefficient for the curves of *S. dysenteriae* growth in the absence of any substance and the curves for *S. dysenteriae* growth in the presence of the substances were calculated and listed as 0.9512, 0.8165, 0.6561, 0.5122, 0.4254, and 0.2975, respectively. These results showed that the curves of the *S. dysenteriae* growth were affected by the concentrations of *R. coptidis* [28].

(iii) Results of PCA. To select the most important variables that could be used to evaluate the quality of the *R.*

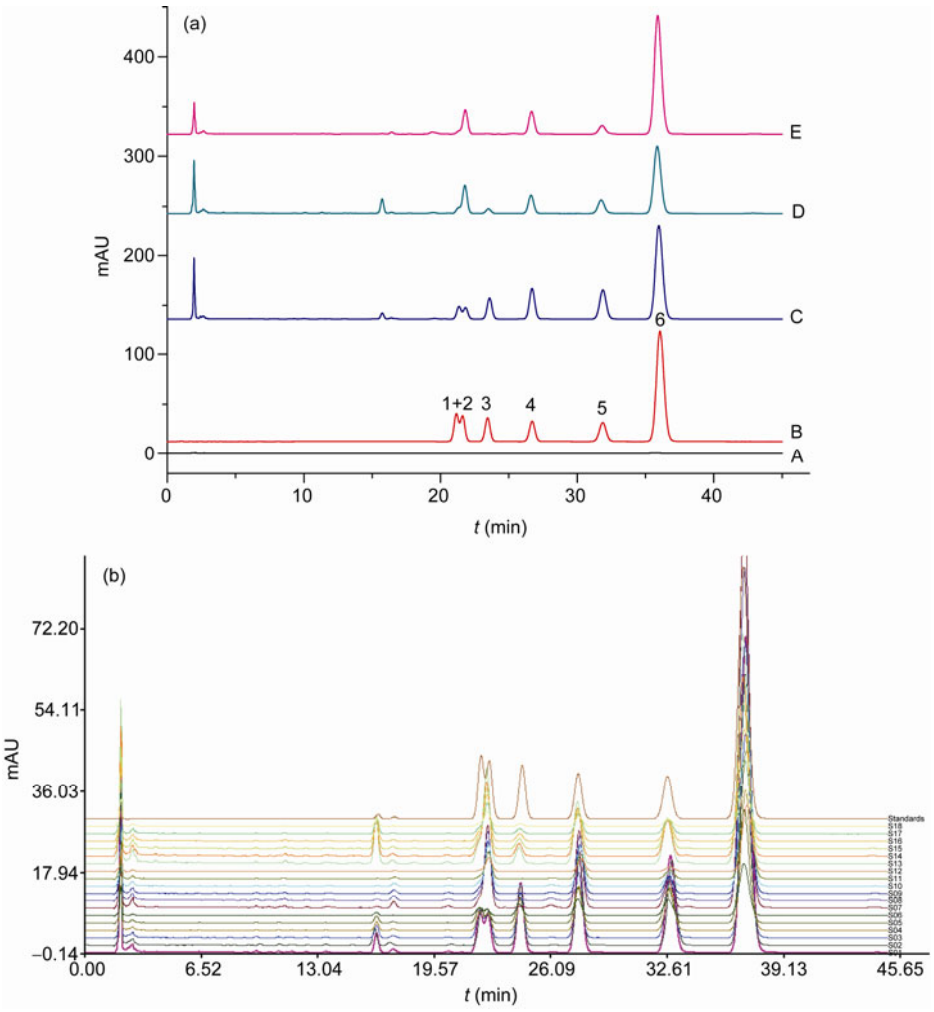

Figure 1 HPLC fingerprints of the multi-source *R. coptidis* and the reference substances. (a) The HPLC fingerprints for (A) methanol, (B) the standards, (C) *Coptis chinensis* Franch, (D) *Coptis deltoidea* C.Y. Cheng et Hsiao, and (E) *Coptis teeta* Wall. Peaks 1–6 correspond to JAT, COL, COP, EPI, PAL, and BER, respectively. (b) The HPLC fingerprints for (S01–06) *Coptis chinensis* Franch., (S07–12) *Coptis deltoidea* C.Y. Cheng et Hsiao, and (S13–18) *Coptis teeta* Wall. From small to big, the order of concentrations of *R. coptidis* were 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 mg mL^{-1}.

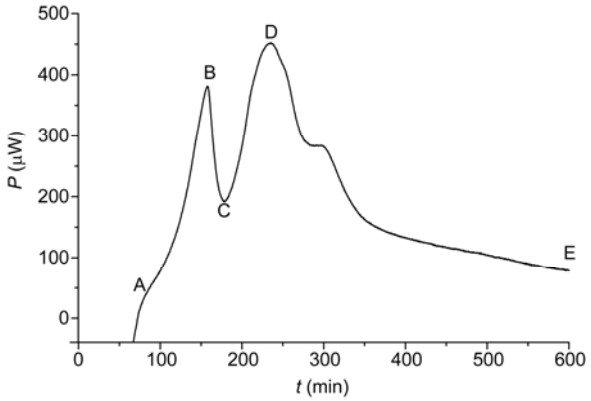

Figure 2 *p-t* curves of *S. dysenteriae* growth at 37°C. The typical metabolic profiles of *S. dysenteriae* culturing in the L.B. culture medium supplemented without any substance were obtained using a 3114 TAM air isothermal calorimeter. They could be divided into four phases: the first exponential growth phase (A-B), a transition phase (B-C), the second exponential growth phase (C-D), and a decline phase (D-E). The biothermokinetic parameters included B (t_1, p_1), D (t_2, p_2), Qt (Area AE), k_1 and k_2 (slope rate from linear equation ln p_t-t of section AB and CD).

coptidis of different sources objectively [29], the PCA of the seven characteristic parameters k_1, k_2, p_1, p_2, t_1, t_2, and Q_t (Table S1) at different concentrations and *R. coptidis* of different sources was performed using the SAS statistical software. On the basis of the eigenvalues being >1, two principal components accounting for 96.72% (the first was 80.63%, and the second was 16.09%) of the total variance were considered to be significant, and a score plot for PCA was shown in Figure 4.

It could be concluded from the score plot of the first two principal components (Figure 4) that parameters t_2 and k_2 could be the two main parameters for evaluating the effect of different concentrations of multi-source *R. coptidis* on *S. dysenteriae* growth. From the values of k_2 and t_2 in Table 1, we could quickly and clearly establish that when t_2 was larger and k_2 was smaller, the growth of *S. dysenteriae* was suppressed to a greater extent.

The 3D histograms of the t_2 and k_2 parameters of different concentrations of multi-source *R. coptidis* were shown

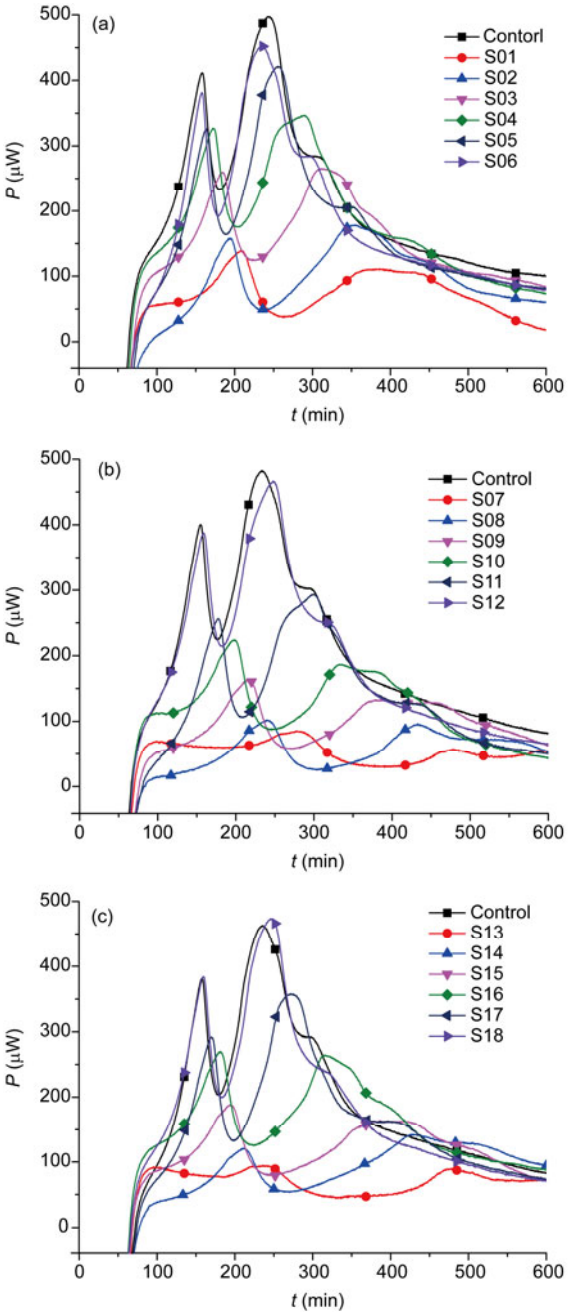

Figure 3 *p-t* curves of S. dysenteriae growth at 37°C affected by different concentrations of the multi-source *R. coptidis*. (a) S01–06 are the different concentrations of *Coptis deltoidea* C.Y. Cheng et Hsiao; (b) S07–12 are the different concentrations of *Coptis teeta* Wall; (c) S13–18 are the different concentrations of *Coptis chinensis* Franch. From top to bottom, the order of concentrations of *R. coptidis* were 0, 0.6, 0.5, 0.4, 0.3, 0.2, and 0.1 mg mL^{-1}.

in Figure 5. Basing on these histograms, it could be concluded that the values of t_2 increased with the increase of the concentration of *R. coptidis*, whereas the values of the other quantitative parameter k_2 decreased in an irregular trend with the increase of the concentration of *R. coptidis*. This phenomenon allowed for the objective and exact evaluation of the effect of *R. coptidis* on *S. dysenteriae* growth, demonstrating that the anti-bacterial effect increased with the concentration. Figure 5 also shows that the anti-bacterial activity of multi-source *R. coptidis* for inhibiting the *S. dysenteriae* growth could be ranked according to the order *CT*>*CC*>*CD*.

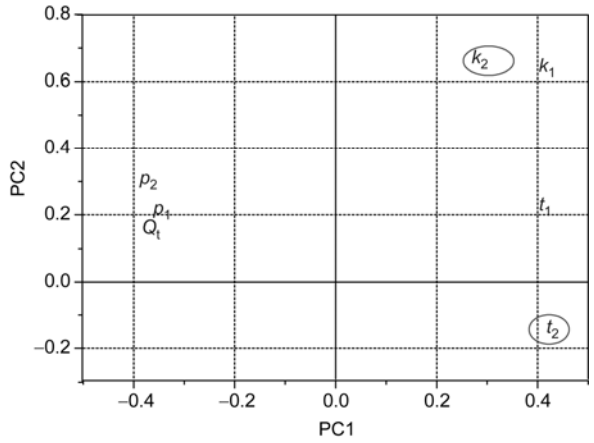

Figure 4 Score plot for PCA. The scatter plot was obtained by PCA on the seven quantitative thermokinetic parameters k_1, k_2, p_1, p_2, t_1, t_2, and Q_t taken from the metabolic *p-t* curves of *S. dysenteriae* growth using software of Origin 8.5 software. The main parameters were marked with circles.

2.3 Results of the combination of CF with BF

To assure the quality of the *R. coptidis* from both the chemical and biological perspectives, the CFs and BFs of different concentrations of multi-source *R. coptidis* were combined to search the internal relationship of "spectrum-activity" and evaluate the anti-bacterial activity of active ingredients in *R. coptidis* using the SAS statistical software. The parameters t_2 and k_2 on behalf of biological aspects were expressed as the first group variables (X_1, X_2), because they were the two main parameters of the seven characteristic parameters according to the PCA. In the chemical aspect, the peak areas of different concentrations of multi-source *R. coptidis* were expressed as the second group variables (Y_1, Y_2, Y_3,…,Y_i, where i is the number of peaks in the CF).

Compared with the CCA result of CFs and BFs, the canonical correlation coefficient r_1 of V_1 and W_1 was 0.9974, whereas the canonical correlation coefficient r_2 of the second pair was 0.9752. And the P values of the two pairs were both less than 0.0001. This result illustrated that the pharmacodynamic results of different concentrations of multi-source *R. coptidis* were closely related to their chemical compositions. The equations used were as follows:

$$\begin{cases} W_1 = 0.7216\,X_1 + 0.9907\,X_2 \\ V_1 = 0.3702\,Y_1 + 0.5045\,Y_2 + 0.3698\,Y_3 + 0.9171\,Y_4 ; \\ \qquad + 0.5695\,Y_5 + 0.9888\,Y_6 \end{cases}$$

Table 1 Quantitative characteristic parameters for *S. dysenteriae* growth at 37°C affected by different concentrations of multi-source *R. coptidis* (n=3)[a)]

	C (mg mL^{-1})	k_1 (min^{-1})	r_1	k_2 (min^{-1})	r_2	t_1 (min)	p_1 (μW)	t_2 (min)	p_2 (μW)	Q_t (J)
	Control	0.0302	0.9999	0.0221	0.9958	157	411.5	235	497.1	5.19
CD	0.6	0.0090	0.9912	0.0074	0.9956	207	139.2	386	111.5	1.63
	0.5	0.0133	0.9994	0.0096	0.9971	193	158.3	354	178.3	2.58
	0.4	0.0161	0.9934	0.0115	0.9992	184	259.6	311	265.1	4.24
	0.3	0.0196	0.9979	0.0158	0.9978	172	327.1	288	346.6	4.95
	0.2	0.0244	0.9992	0.0197	0.9991	163	326.8	255	421.5	5.37
	0.1	0.0287	0.9971	0.0228	0.9974	155	381.4	235	452.2	6.05
CT	0.6	0.0042	0.9918	0.0037	0.9985	282	81.6	481	53.9	0.83
	0.5	0.0081	0.9926	0.0052	0.9948	243	97.3	436	90.5	1.37
	0.4	0.0130	0.9933	0.0081	0.9983	221	155.1	392	124.7	2.00
	0.3	0.0158	0.9978	0.0125	0.9976	202	210.8	335	171.8	2.67
	0.2	0.0215	0.9969	0.0158	0.9991	179	243.8	299	274.6	3.36
	0.1	0.0259	0.9974	0.0186	0.9992	162	369.1	252	437.3	4.80
CC	0.6	0.0080	0.9982	0.0062	0.9926	235	114.6	465	107.4	1.09
	0.5	0.0129	0.9974	0.0081	0.9993	210	96.0	420	114.8	2.05
	0.4	0.0154	0.9932	0.0099	0.9973	193	188.3	374	160.3	3.13
	0.3	0.0193	0.9979	0.0145	0.9970	180	269.8	313	258.4	3.67
	0.2	0.0246	0.9990	0.0179	0.9993	169	316.8	270	374.9	4.25
	0.1	0.0281	0.9960	0.0216	0.9974	159	364.0	244	442.8	5.26

a) *CD*, different concentrations of *Coptis deltoidea* C.Y. Cheng et Hsiao. *CT*, different concentrations of *Coptis teeta* Wall. *CC*, different concentrations of *Coptis chinensis* Franch.

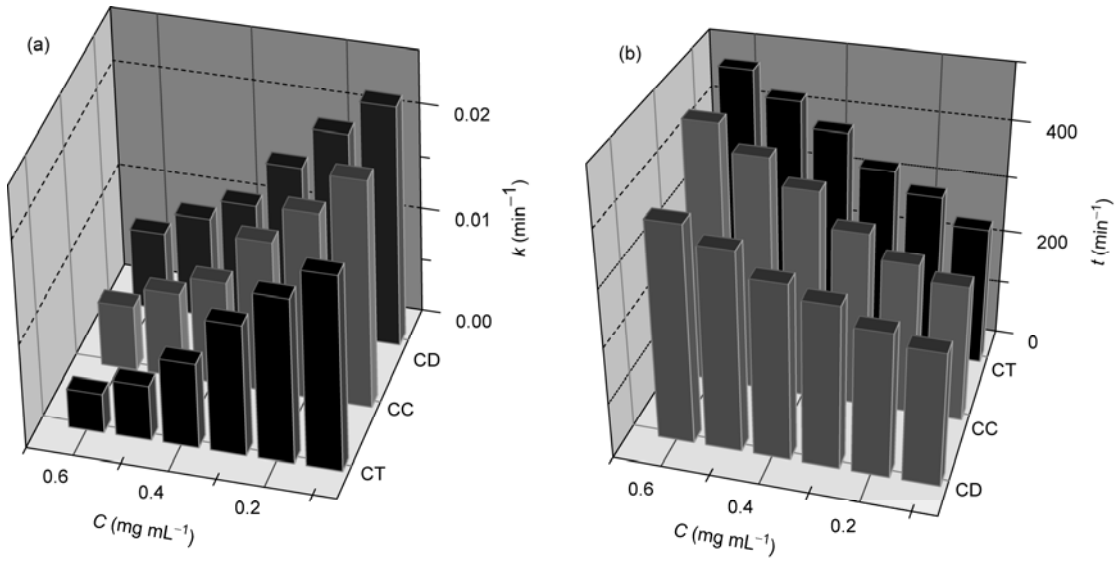

Figure 5 Relationship between the quantitative thermokinetic parameters and the different concentrations of the *R. coptidis* of different sources. The column maps of the two quantitative thermokinetic parameters that contributed to the PCA to the greatest extent (a) the growth rate constants k_2, (b) the appearance times of the maximum power output t_2 were performed using the software of Origin 8.5.

$$\begin{cases} W_2 = 0.6733\,X_1 + 0.1134\,X_2 \\ V_2 = 0.0749\,Y_1 + 0.5317\,Y_2 + 0.2860\,Y_3 + 0.2423\,Y_4, \\ \quad + 0.3972\,Y_5 + 0.0311\,Y_6 \end{cases}$$

where the first and the second pairs of canonical variables were expressed as W_1, V_1 and W_2, V_2, respectively. COL, JAT, EPI, COP, PAL and BER were expressed as X_1–X_6, respectively. It could be concluded from the equation that the anti-bacterial action of *R. coptidis* appeared to be broadly related to JAT, COP, PAL and BER. The bacteriostatic effect of *R. coptidis* was not significantly affected by COL and EPI. This could be particularly useful for predicting the potential efficacy of the substances in the herbal medicines. With further development, the CFs could be used to clarify the general differences in the chemical compositions of herbal medicines of different sources. Similarly, BFs could be used to calibrate/supplement/magnify the biological characteristics of multi-source herbal medicines. Compared with QC standards for herbal medicines, the combination of BFs and CFs could allow for the generation of more comprehensive QC data. It could also be applied as an effective complementary method for the QC of other multi-source medical plants.

3 Conclusions

In this study, we have successfully evaluated the effect of different concentrations of multi-source *R. coptidis* on *S. dysenteriae* growth by combining CFs with BFs. The results in this study of the PCA of BFs showed that the bacteriostatic effects enhanced with the increase of the concentration, which was consistent with the preliminary experiment reported in the literature [29]. And the anti-bacterial activity of *R. coptidis* of different sources was found to be of the order *CT*>*CC*>*CD*.

The results of the similarity analyses of CFs indicated that there were obvious differences in the alkaloid varieties and contents among the *R. coptidis* of different sources. When we combined the CFs with BFs, the results could be acquired easily that the anti-bacterial action of *R. coptidis* appeared to be broadly related to JAT, COP, PAL and BER. This combination could be used to search the internal relationship of "spectrum-activity" and evaluate active ingredients of *R. coptidis* for antimicrobials. Hence, the use of such a combination would make it easier to predict the potentially pharmacodynamic materials in herbal medicines and assure the quality of multi-source medical plants.

This study introduced a method for the QC of multi-source medical plants. The data provided in this study also showed the potential applicability and development prospects of the approach combining CFs with BFs. This method could be used to assure the quality of multi-source medical plants. The application of bioanalytical methods can seek out active substances based on their biological effects, and therefore overcome the seemingly random and currently poorly defined expectations in QC. The extent to which bioanalytical methods can be applied, however, is relatively narrower than that of the chemical analysis methods because of the lack of maneuverability and reproducibility. For actual researches, active substances can be identified by combining the CF with BF techniques. And the active substances can then be measured using the chemical analysis methods to evaluate the quality of the products. Compared with the currently available analytical methods of chemistry, our approach is more scientific and explicit.

This work was supported by the National Natural Science Foundation of China (81274078), the Beijing Natural Science Foundation (7112128) and the Open Research Fund of the State Key Laboratory Breeding Base of Systematic Research, Development and Utilization of Chinese Medicinal Resources.

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